

T4 DNA Polymerase

(Bacteriophage T4 of *Escherichia coli*)

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Cat. No.	Size
E1100-01	200 units
E1100-02	1 000 units

Unit Definition:

One unit is the amount of enzyme that catalyzes the incorporation of 10 nmoles of total nucleotide into acid-insoluble product in 30 min at 37°C.

Storage Conditions:

Store at -20°C

T4 DNA Polymerase is a mesophilic polymerase, exhibiting very strong 3'→5' exonuclease activity.

Description:

- Exhibits 5'→3' polymerase and 3'→5' exonuclease activities (1, 2).
- Adds labeled nucleotides to the recessed 3'-ends of DNA fragments.
- The polymerase requires the presence of a single-stranded DNA template and a primer.
- Exonuclease, stronger than that found in DNA Polymerase I, is more active on single-stranded DNA than on double-stranded DNA.
- Ultrapure recombinant enzyme.
- Exonuclease activity can be used to remove one or a few nucleotides from 3'-end of double-stranded DNA.
- Enzyme suitable for:
 - › 3' overhang removal to form blunt ends (3,4)
 - › 5' fill-in to form blunt ends (3,4)
 - › probe labeling using replacement synthesis (3,4)
 - › single strand deletion subcloning (5)
 - › second strand synthesis in site-directed mutagenesis (6)

Storage Buffer:

20 mM potassium phosphate (pH 6.5), 5 mM dithiothreitol and 50% (v/v) glycerol.

Assay Conditions:

67 mM Tris-HCl (pH 8.8 at 22°C), 6.7 mM MgCl₂, 10 mM dithiothreitol, 16.6 mM ammonium sulfate, 6.7 μM EDTA, 20 μg bovine serum albumin, 45 μg activated calf thymus DNA and 0.033 mM each of dCTP, dGTP, dTTP and [α -³²P]dATP. Incubation is at 37°C for 30 min in a reaction volume of 100 μl (1).

Quality Control:

All preparations are tested for contaminating endonuclease activity.

References:

1. Goulian, M., Lucas, Z.J. and Kornberg, A. (1968) *J. Biol. Chem.* 243, 627-638.
2. Lehman, I.R. (1981) *Enzymes* 14, 51-65.
3. Tabor, S. and Struhl, K (1989) in *Current Protocols in Molecular Biology* (Ausubel, F. M., et al., eds) pp. 3.5.10-3.5.12, John Wiley&Sons, New York.
4. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual, second edition*, pp. 5.44-5.47, Cold Spring Harbour.
5. Dale, R., McClure, B. and Houchins, J., (1985) *Plasmid* 13, 31-40.
6. Kunkel, T. A., Roberts, J. D. and Zakour, R. A. (1987) *Methods Enzymol.* 154, 367-382.